ATCC

Product Sheet

RWPE2-W99 (ATCC® CRL-2853™)

Please read this FIRST

Storage Temp.
liquid nitrogen vapor phase

Biosafety Level
2

Intended Use

This product is intended for research use only. It is not intended for any animal or human therapeutic or diagnostic use.

Complete Growth Medium

The base medium for this cell line is provided by Invitrogen (GIBCO) as part of a kit: Keratinocyte Serum Free Medium (K-SFM), Kit Catalog Number 17005-042. This kit is supplied with each of the two additives required to grow this cell line (bovine pituitary extract (BPE) and human recombinant epidermal growth factor (EGF). To make the complete growth medium, you will need to add the following components to the base medium:

- 0.05 mg/ml BPE - provided with the K-SFM kit
- 5 ng/ml EGF - provided with the K-SFM kit. NOTE: Do not filter complete medium.

Citation of Strain

If use of this culture results in a scientific publication, it should be cited in that manuscript in the following manner: RWPE2-W99 (ATCC® CRL-2853™)

Description

Organism: *Homo sapiens*, human
Tissue: prostate
Disease: normal
Cell Type: epithelial
Age: 54 years
Gender: male
Morphology: epithelial
Growth Properties: adherent
DNA Profile:
Amelogenin: X,Y
CSF1PO: 13
D13S317: 8,14
D16S539: 9,11
D5S818: 12,15
D7S820: 10,11
THO1: 8,9.3
TPOX: 8,11
vWA: 14,18
Cytogenetic Analysis: The depositor reports that at passage 44, a majority of the cells had a near diploid chromosome number of 48; X, -Y.

Batch-Specific Information

Refer to the Certificate of Analysis for batch-specific test results.

SAFETY PRECAUTION

ATCC highly recommends that protective gloves and clothing always be used and a full face mask always be worn when handling frozen vials. It is important to note that some vials leak when submersed in liquid nitrogen and will slowly fill with liquid nitrogen. Upon thawing, the conversion of the liquid nitrogen back to its gas phase may result in the vessel exploding or blowing off its cap with dangerous force creating flying debris.

Unpacking & Storage Instructions

1. Check all containers for leakage or breakage.
2. Remove the frozen cells from the dry ice packaging and immediately place the cells at a temperature below -130°C, preferably in liquid nitrogen vapor, until ready for use.

Handling Procedure for Frozen Cells

Handling Procedure for Frozen Cells

To insure the highest level of viability, thaw the vial and initiate the culture as soon as possible upon receipt. If upon arrival, continued storage of the frozen culture is necessary, it should be stored in liquid nitrogen vapor phase and not at -70°C. Storage at -70°C will result in loss of viability.

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1. Thaw the vial by gentle agitation in a 37°C water bath. To reduce the possibility of contamination, keep the O-ring and cap out of the water. Thawing should be rapid (approximately 2 minutes).
2. Remove the vial from the water bath as soon as the contents are thawed, and decontaminate by dipping in or spraying with 70% ethanol. All of the operations from this point on should be carried out under strict aseptic conditions.
3. Transfer the vial contents to a centrifuge tube containing 9.0 ml complete culture medium and spin at approximately 125 x g for 5 to7 minutes.
4. Resuspend cell pellet with the recommended complete medium (see the specific batch information for the culture recommended dilution ratio) and dispense into a 25 cm^2 or a 75 cm^2 culture flask. It is important to avoid excessive alkalinity of the medium during recovery of the cells. It is suggested that, prior to the addition of the vial contents, the culture vessel containing the complete growth medium be placed into the incubator for at least 15 minutes to allow the medium to reach its normal pH (7.0 to 7.6).
5. Incubate the culture at 37°C in a suitable incubator. A 5% CO_2 in air atmosphere is recommended if

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using the medium described on this product.

Subculturing Procedure

Protocol:

1. Remove and discard culture medium.
2. Briefly rinse the cell layer with Ca++/Mg++ free Dulbecco's phosphate-buffered saline (D-PBS).
3. Add 2.0 to 3.0 ml (to a T-25 flask) or 3.0 to 4.0 ml (to a T-75 flask) of 0.05% Trypsin - 0.53 mM EDTA solution, diluted 1:1 with D-PBS, and place flask in a 37C incubator for 5 to 8 minutes. Observe cells under an inverted microscope until cell layer is dispersed (usually within 5 to 10 minutes).
   Note: To avoid clumping do not agitate the cells by hitting or shaking the flask while waiting for the cells to detach.
4. Add 6.0 to 8.0 ml of 0.1% Soybean Trypsin Inhibitor (or 2% fetal bovine serum in D-PBS), as appropriate, and aspirate cells by gently pipetting.
5. Transfer cell suspension to centrifuge tube and spin at approximately 125 x g for 5 to 7 minutes.
6. Discard supernatant and resuspend cells in fresh serum-free growth medium. Add appropriate aliquots of cell suspension to new culture vessels. An inoculum between 2 X 10(4) to 4 X 10(4) viable cells/sq. cm is recommended.
7. Incubate cultures at 37C. We recommend that you maintain cultures at a cell concentration between 4 X 10(4) and 8 X 10(4) cells/sq. cm.

Cells grown under serum-free or reduced serum conditions may not attach strongly during the 24 hours after subculture and should be disturbed as little as possible during that period.
Subcultivation Ratio: A subcultivation ratio of 1:3 to 1:5 is recommended
Medium Renewal: Every 48 hours

Cryopreservation Medium

Cryoprotectant Medium

Complete growth medium described above supplemented with 15% fetal bovine serum and 10% (v/v) DMSO.

Cell culture tested DMSO is available as ATCC® Catalog No. 4-X.

Comments

The RWPE2-W99 cell line was derived, in 1999, from the RWPE-2 (ATCC CRL-11610) cell line by cloning in soft agar to select cells that show high expression of Ki-ras. RWPE2-W99 cells show strong expression of Ki-ras and have characteristics similar to those of RWPE-2 cells. RWPE-2 cells were derived from RWPE-1 cells (ATCC CRL-11609) by transformation with Ki-ras using the Kirsten murine sarcoma virus (Ki-MuSV) to establish the RWPE-2 cells (PubMed: 9214605). Epithelial cells from the peripheral zone of a histologically normal adult human prostate were transfected with a plasmid carrying one copy of the human papilloma virus 18 (HPV-18) genome to establish the RWPE-1 cell line (ATCC CRL-11609). A family of tumorigenic cell lines that mimics multiple steps in prostate cancer progression was also derived from RWPE-1 cells by exposure to N-methyl-N-nitrosourea (MNU): WPE1-NA22 (ATCC CRL-2849), WPE1-NB14 (ATCC CRL-2850), WPE1-NB11 (ATCC CRL-2851) and WPE1-NB26 (ATCC CRL-2852).
The depositor reports that this cell line was screened for Hepatitis B and C, and human immunodeficiency viruses, and was found to be negative.

References

References and other information relating to this product are available online at www.atcc.org.

Biosafety Level: 2

Appropriate safety procedures should always be used with this material. Laboratory safety is discussed in the current publication of the *Biosafety in Microbiological and Biomedical Laboratories* from the U.S. Department of Health and Human Services Centers for Disease Control and Prevention and National Institutes for Health.

ATCC Warranty

ATCC® products are warranted for 30 days from the date of shipment, and this warranty is valid only if the product is stored and handled according to the information included on this product information sheet. If the ATCC® product is a living cell or microorganism, ATCC lists the media formulation that has been found to be effective for this product. While other, unspecified media may also produce satisfactory results, a change in media or the absence of an additive from the ATCC recommended media may affect recovery, growth and/or

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function of this product. If an alternative medium formulation is used, the ATCC warranty for viability is no longer valid.

Disclaimers

This product is intended for laboratory research purposes only. It is not intended for use in humans.
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Additional information on this culture is available on the ATCC web site at www.atcc.org.
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